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Award Number: DAMD17-01-1-0416

TITLE: Novel Drug Delivery Technique for Breast Cancer Therapy

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REPORT DATE: July 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Annual (1 Jul 01 - 30 Jun 02)

4. TITLE AND SUBTITLE
Novel Drug Delivery Technique for Breast Cancer Therapy

5. FUNDING NUMBERS
DAMD17-01-1-0416

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

This report describes the progress achieved during the first year of the project. We proposed to implement Task 1 (a and b) and Task 2a in the first year of the project. Task 1 (a and b) focuses on in vivo studies of ultrasound-induced penetration of model (fluorescent) anti-cancer drugs in human MCF-7 breast tumors of nude mice. Task 2a is devoted to in vivo studies of ultrasound-induced penetration of real anti-cancer drug 5-FU in the breast tumors. We conducted these studies to implement the proposed tasks. Our data obtained with regular and fluorescence microscopy indicate that the ultrasound produces enhanced delivery of model and real anti-cancer drugs in the tumors. We demonstrated increased penetration of the fluorescent anti-cancer drug from blood into ultrasound-irradiated tumors compared with control (non-irradiated) tumors. Dramatic tumor necrosis was obtained when mice injected with real anti-cancer drug and the tumors were irradiated by ultrasound, while no tumor necrosis was noticed when the drug was used without irradiation. Our data suggest that the proposed novel technique may improve efficacy of breast cancer chemotherapy.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast cancer, drug delivery systems, multi-drug resistance, chemotherapy	9
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

Breast cancer is a major health problem in the USA. Despite the recent progress in the development of promising anti-cancer chemo- and biotherapeutic agents, no breakthrough has been achieved in breast tumor therapy and therapy of tumors in other organs. The efficacy of anti-cancer drugs (especially most promising macromolecular agents) is limited due to their poor penetration through tumor capillary walls, interstitium, and cancer cell membranes [1, 2]. The objective of the proposed research is to develop and test a novel drug delivery technique utilizing interaction of ultrasound with exogenous microparticles that can selectively accumulate in tumors [3]. The interaction of ultrasound with the microparticles results in cavitation (formation, growth, and collapse of microbubbles) in tumors without damage to normal tissues. The microparticles serve as cavitation nuclei that substantially lower cavitation threshold selectively in tumors [4-6]. The ultrasound-induced cavitation perforates tumor blood vessel walls and cancer cell membranes and induces microconvection in the interstitium that enhances penetration of anti-cancer drugs in the cancer cells. The focus of this research project is to study ultrasound-enhanced delivery of model and real drugs in human breast tumors and perform breast cancer chemo- and biotherapy by using the ultrasound-enhanced drug delivery. The studies are performed *in vivo* in nude mice bearing human MCF-7 breast tumors. The first year of the project focuses on the studies of ultrasound-enhanced delivery of model (dextrans with fluorescence) and real (5-FU) anti-cancer drugs in the breast tumors.

BODY

Materials and Methods

The studies were performed in athymic nude mice (average weight of 30 g). These studies were approved by the Institutional Animal Care and Use Committee (IACUC) of UTMB.

Suspensions of human breast MCF-7 cancer cells (15×10^6 per site) were injected s.c. in the dorso-scapular area on the left and right sides of each mouse. Experiments were initiated when tumors reached the size of 5 to 8 mm.

One tumor was irradiated by 20-kHz ultrasound for 10 minutes by pulses with duration of 0.1 s and repetition rate of 2 Hz, while the other tumor served as control. Ultrasound pressure amplitude measured by a calibrated hydrophone was 4 bar. Ultrasound with these parameters provides efficient cavitation in tissue phantoms with polystyrene nanoparticles [5].

Polystyrene nanoparticles (10% w/w in water, dia. = 100 nm) were used as cavitation nuclei. The nanoparticles were injected in the tail vein of nude mice one day prior to irradiation to allow extravasation of the particles in tumor blood vessels. In the experiment with fluorescent model anti-cancer drug we also used polystyrene nanoparticles colored with FITC (which has green fluorescence) to visualize distribution of the nanoparticles in the tumors.

Rhodamine-dextrans (which have red fluorescence) were used model anti-cancer agents. Thin (5-μm) sections of the irradiated and control tumors were studied with a fluorescence microscope to visualize distribution of rhodamine-dextrans. After fluorescence studies immunohistochemical staining of these sections with CD31 was performed to visualize tumor blood vessels. Same areas of tumors were found with the microscope under regular illumination and photographed for comparison with the fluorescence data.

5-Fluorouracil (5-FU) was used as an anti-cancer agent. This drug is being used for chemotherapy in patients and in cancer studies in nude mice. 5-FU was injected i.p. 1 minute prior to irradiation at the dose of 90 mg/kg (typical dose for studies in mice). Histopathologic evaluation of the tumors with standard H&E staining was performed for mice after treatment with 5-FU to assess tumor necrosis.

Results

Figure 1a shows regular microscopy of a thin slice of a control (non-irradiated) MCF-7 tumor. There is a tumor blood vessel in the central part of the picture. Examination of the same area of the tumor by fluorescent microscopy with a green filter suitable for visualization of FITC-stained nanoparticles (Fig. 1b) demonstrated that the fluorescent nanoparticles are accumulated in the blood vessel. Fluorescence microscopy of this area with a red filter suitable for visualization of rhodamine distribution (Fig. 1c) shows that the rhodamine-dextran (M.W. = 70,000 Da) molecules accumulated mostly in the blood vessel and do not penetrate in the tumor interstitium.

To visualize the tumor blood vessel, we stained the tumor slices with CD31. Fig. 1d confirms that the blood vessel (stained by CD31 in brown color) is in the center of this tumor area. By using a special computer data processing algorithm we combined pictures presented in Figures 1 (c and d) to demonstrate the distribution of the rhodamine-dextran with respect to the tumor blood vessel. Fig. 1e represents this combination that confirms that the rhodamine-dextran molecules accumulated mostly in the blood vessel and do not penetrate in the tumor interstitium.

Similar fluorescent and immunohistochemical studies were conducted for the ultrasound-irradiated tumors. Figure 2a shows regular microscopy of a thin slice of the irradiated MCF-7 tumor of the same mouse. Examination of the same area of the tumor by fluorescent microscopy with the green filter (Fig. 2b) demonstrated that the fluorescent nanoparticles are distributed in the tumor. Fluorescence microscopy of this area with the red filter (Fig. 2c) shows that the rhodamine-dextran molecules penetrated in the tumor interstitium. Fig. 2d shows that the tumor blood vessels (stained by CD31) are damaged. The combination of Figures 2 (c and d) demonstrated that the rhodamine-dextran molecules penetrate in the tumor interstitium from blood vessels.

To study tumor necrosis induced by enhanced delivery of anti-cancer drug 5-FU by ultrasound, we divided mice in three groups. The first group of mice was not injected with 5-FU; the second group was injected with 5-FU only; and the third group was injected with 5-FU and polystyrene nanoparticles. Ultrasound was applied to one of the tumors of each mouse.

Histological examination of the breast MCF-7 tumor slices stained with H&E revealed: viable tumor cells with well-defined nuclei in control tumors (no ultrasound, no drug injection) (Fig. 3a); necrosis in tumors of the same mouse irradiated by ultrasound in combination with nanoparticle injection (Fig. 3b); very minor necrosis produced by 5-FU in non-irradiated tumors (Fig. 4a); necrosis in irradiated tumors produced by 5-FU in combination with ultrasound (Fig. 4b); dramatic necrosis in large areas when ultrasound was used in combination with 5-FU and nanoparticles (Fig. 5b), while no necrosis was induced by 5-FU without ultrasound irradiation (Fig. 5a).

KEY RESEARCH ACCOMPLISHMENTS

1. We studied ultrasound-enhanced delivery of model fluorescent anti-cancer drugs in human breast tumors of nude mice. Our studies performed with fluorescence microscopy demonstrated that ultrasound-induced cavitation substantially improves delivery of the model anti-cancer drug from blood into the breast tumors through the tumor blood vessels.
2. We studied ultrasound-enhanced delivery of real anti-cancer drug (FU-5, widely used for cancer therapy) in human breast tumors of nude mice. Histological examination of the ultrasound-irradiated and control tumors indicated that: (a) no necrosis produced by the anti-cancer drug in the control, non-irradiated tumors; and (b) irradiation of tumors by ultrasound performed with 5-FU injections resulted in dramatic tumor necrosis and death of cancer cells in the tumors.

The results of these studies suggest that this novel drug delivery technique may substantially improve efficacy of the breast cancer chemotherapy. Our next studies planned for the second and third years of the project will show the optimum conditions for ultrasound-enhanced anti-cancer drug delivery in tumors and efficacy of the breast cancer chemotherapy with real anti-cancer drugs 5-FU and Interleukin-2.

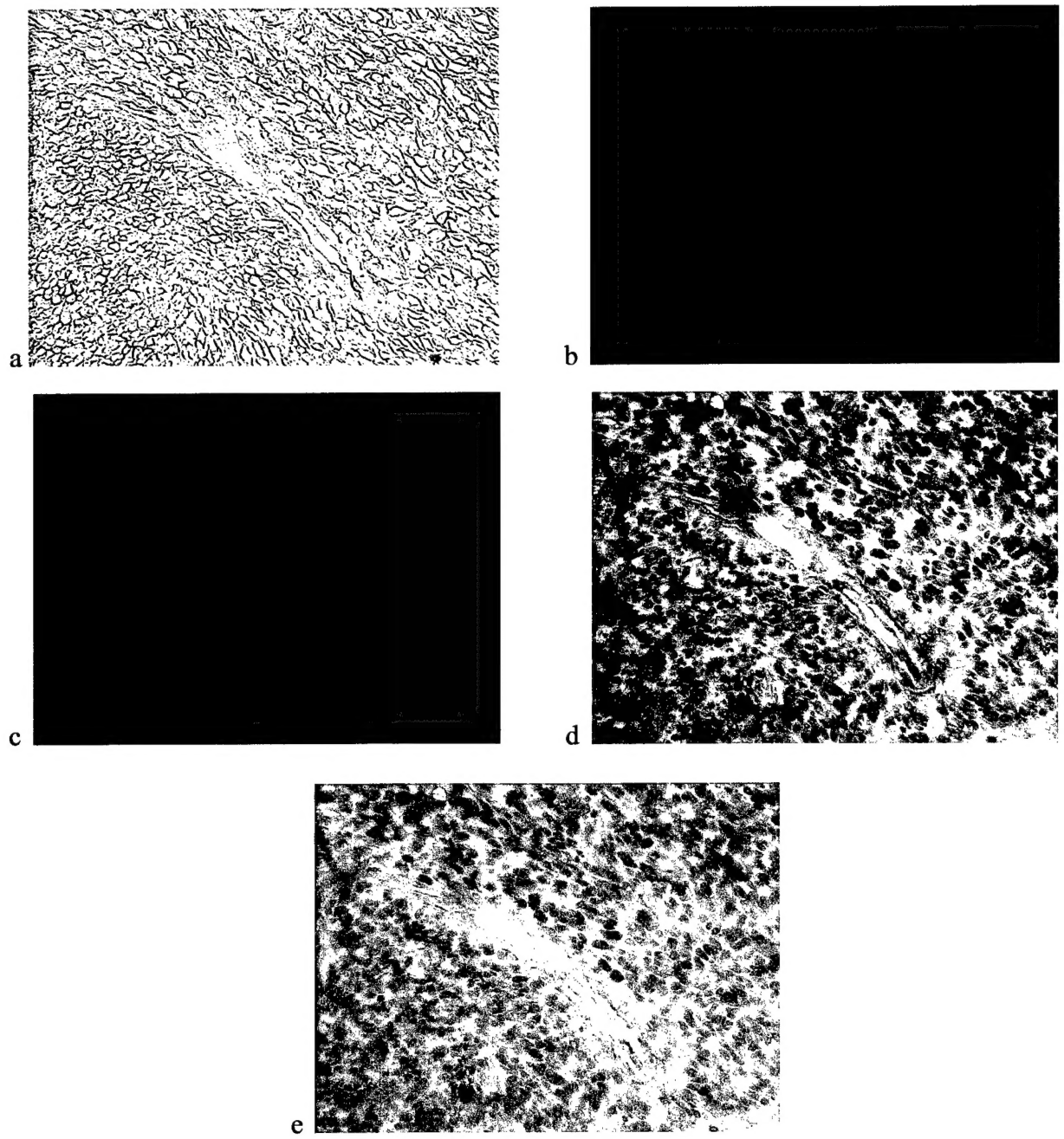

Figure 1. Microscopic examination of a control (non-irradiated) MCF-7 breast tumor of a nude mouse: (a) regular microscopy; (b) fluorescence microscopy with the green filter showing the polystyrene nanoparticle distribution; (c) fluorescence microscopy with the red filter showing rhodamine-dextran distribution; (d) immunohistostaining of blood vessels of the control tumor with CD31; (e) combination of c and d showing distribution of rhodamine-dextran with respect to the tumor blood vessels.

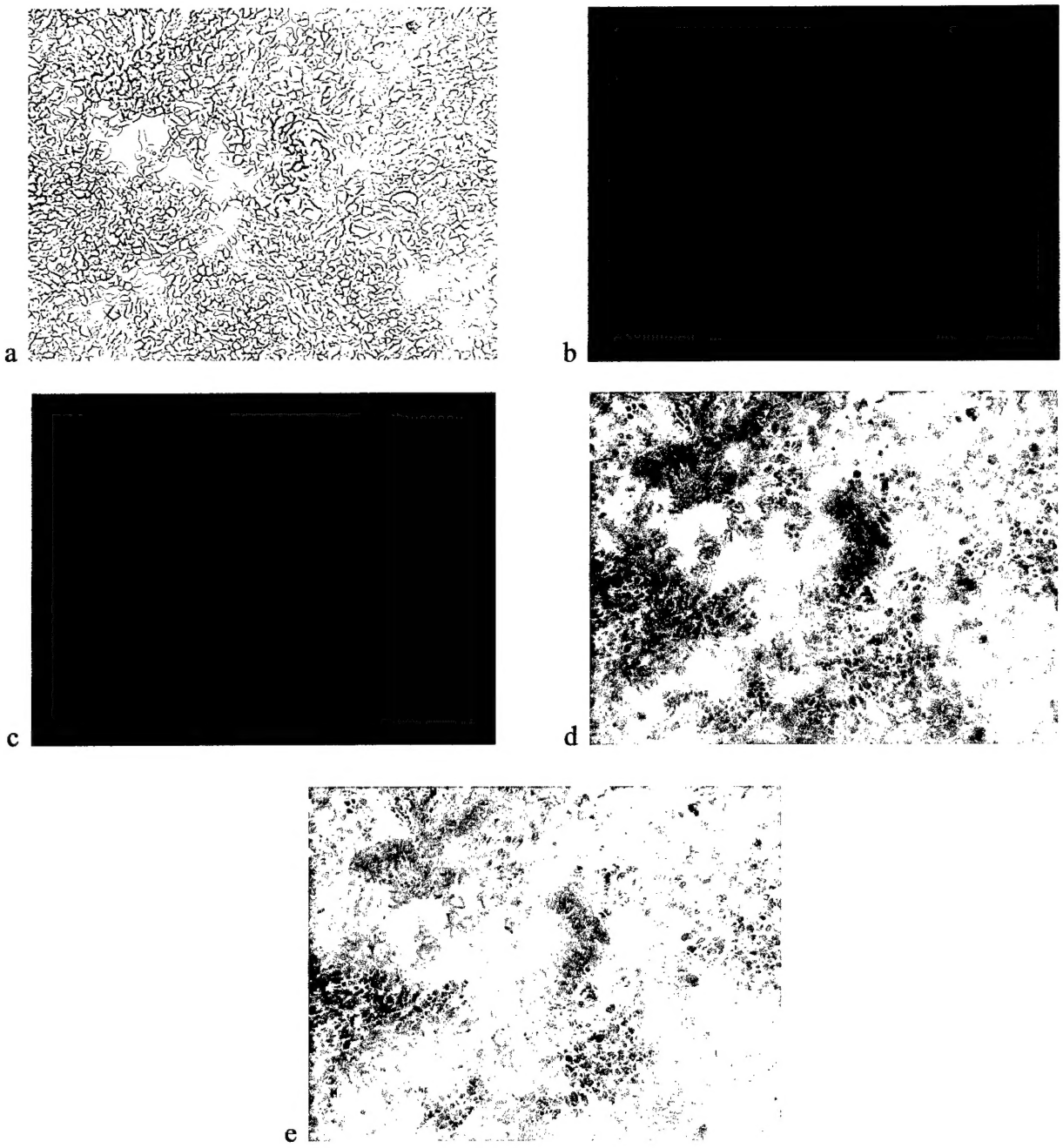

Figure 2. Microscopic examination of the irradiated MCF-7 breast tumor of the same nude mouse: (a) regular microscopy; (b) fluorescence microscopy with the green filter showing the polystyrene nanoparticle distribution; (c) fluorescence microscopy with the red filter showing rhodamine-dextran distribution; (d) immunohistostaining of blood vessels of the control tumor with CD31; (e) combination of c and d showing distribution of rhodamine-dextran with respect to the tumor blood vessels.

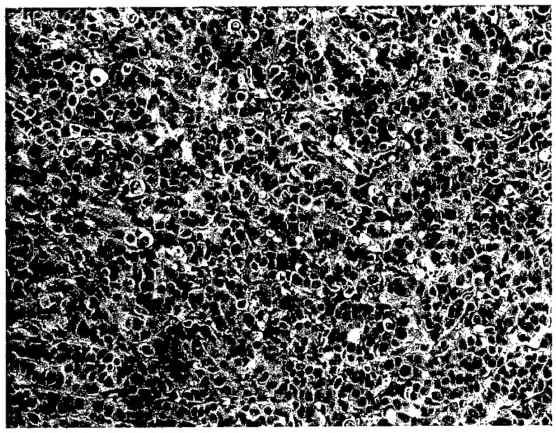

Figure 3a. Control tumor of a mouse (no drug injection, no ultrasound irradiation).

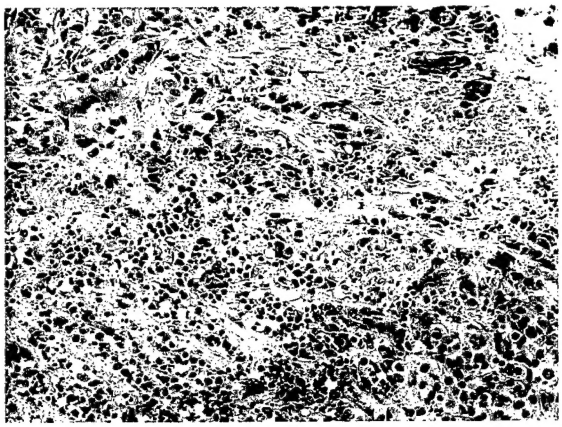

Figure 3b. Irradiated tumor of the same mouse (no drug injection, ultrasound irradiation with nanoparticle injection).

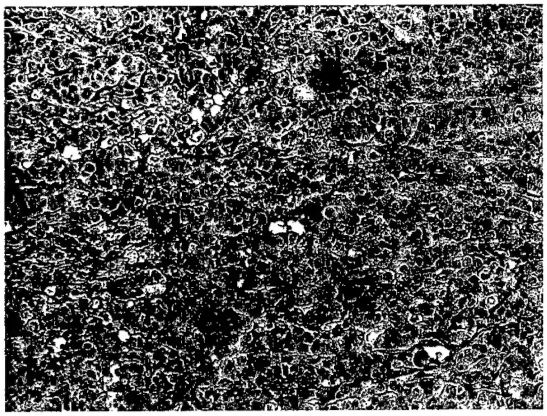

Figure 4a. Control tumor of a mouse (drug injection, no ultrasound irradiation).

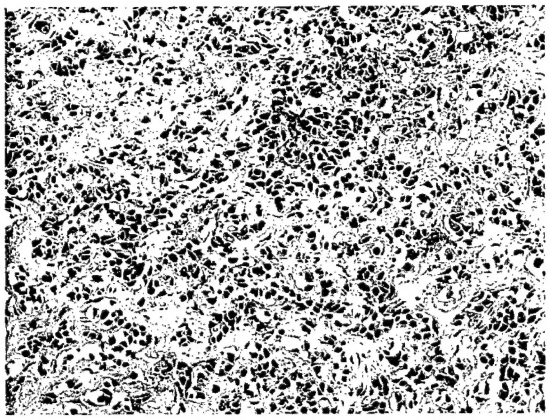

Figure 4b. Irradiated tumor of the same mouse (drug injection, ultrasound irradiation, no nanoparticle injection).

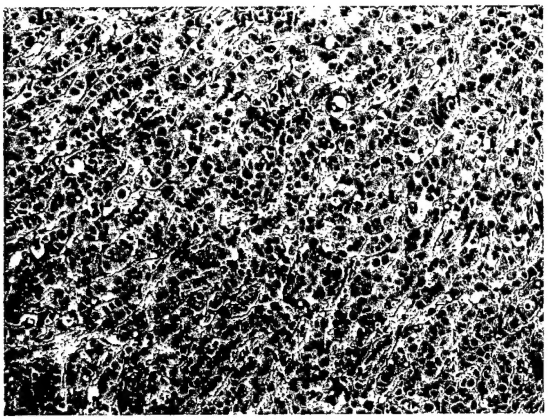

Figure 5a. Control tumor of a mouse (drug injection, no ultrasound irradiation).

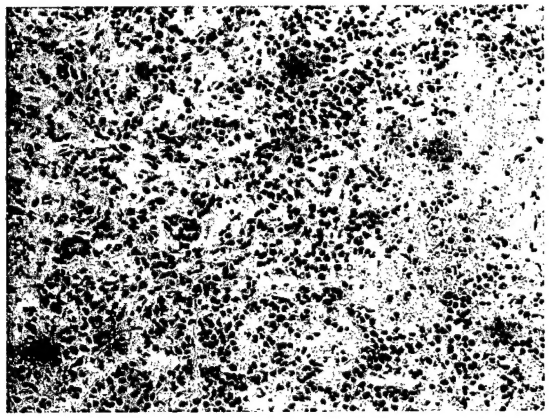

Figure 5b. Irradiated tumor of the same mouse (drug injection, ultrasound irradiation with particle injection).

REPORTABLE OUTCOMES

We have submitted two 2-page summaries to the Second Joint Conference of the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society:

1. Y. Ivanova, B. M. Evers, R. Thomas, T. V. Ashitkov, R. O. Esenaliev. Nanoparticles and Ultrasound for Delivery of Model Macromolecular Anti-cancer Drugs in Tumors. Second Joint Conference of the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society, Houston, TX, October, 2002.
2. I. V. Larina, B. M. Evers, C. Bartels, T. V. Ashitkov, K.V. Larin, R. O. Esenaliev. Ultrasound-enhanced Drug Delivery for Efficient Cancer Therapy. Second Joint Conference of the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society, Houston, TX, October, 2002.

These summaries were accepted for presentation at the conference by the Conference Committee. The conference will be in Houston, TX in October 2002. The reprints of the summaries will be sent to the U.S. Army Medical Research and Material Command after publication of the summaries in the Conference Proceedings in October, 2002.

CONCLUSIONS

Our studies demonstrated that interaction of ultrasound with nanoparticles results in enhanced delivery of model anti-cancer drugs in the tumor interstitium. Histological examinations of tumors after irradiation with ultrasound in combination with 5-FU and polystyrene nanoparticles injections shows dramatic necrosis produced by ultrasound-enhanced delivery of 5-FU in tumor tissue. This result demonstrates that interaction of ultrasound radiation with nanoparticles may substantially improve the effect of anti-cancer drug 5-FU on breast tumor.

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